

Title: Ceramide Kinase Regulates TNF- α -Induced Immune Responses in Human Monocytic Cells

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Supplementary Figure 1

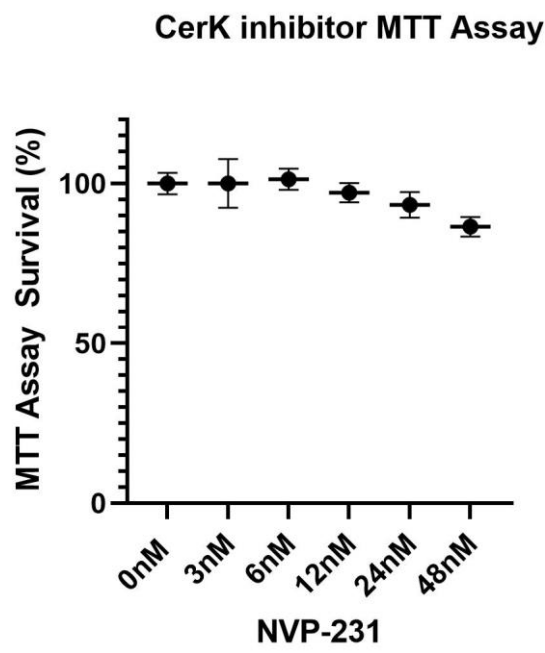


Figure S1. Cell viability assay

The cell viability was detected by MTT assay (TACS#4890-25-01, R&D systems, USA) as per manufacturer instructions. Briefly, THP-1 cells were plated in a 96-well plate at a density of 2×10^5 cells/ml, with 100 μ l per well. Cells were treated with different concentrations of CERK inhibitor NVP-231 for 1h. Then 10 μ l of MTT solution was added into each well. Cells were incubated at 37 °C for 4 h. 100 μ l of solubilization solution (10% SDS in 0.01M HCL) was added to dissolve the formazan crystals. The optical density was measured at 570 nm and blanked at 650 nm (Synergy H4, BioTek, USA). Cell viability was calculated as the percentage of surviving fraction after exposure to NVP-231 relative to the mock control. All data are expressed as mean \pm SEM (n=6).

Supplementary Figure.2

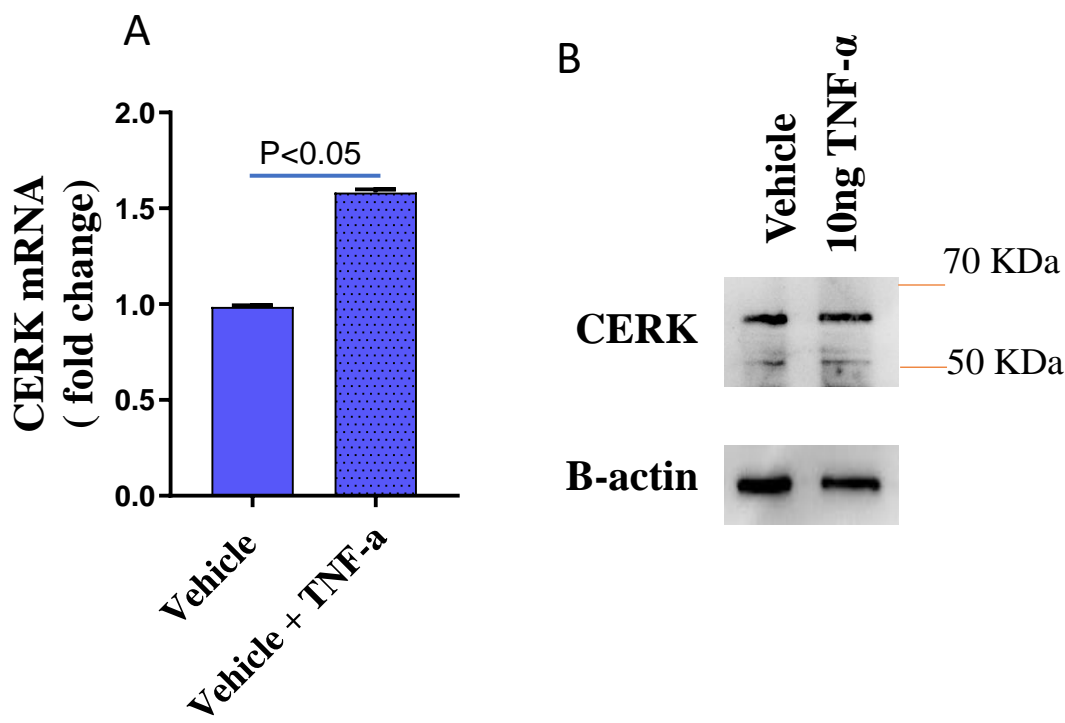


Figure S2 . Impact of TNF- α on CERK expression at both gene and protein levels in monocytic cells. Monocytic cells were treated with vehicle or TNF- α vehicle for 4 hours. **(A)** Gene expression of CERK was determined by real time RT-PCR. **(B)** For protein expression, cells were treated with vehicle or TNF- α for 12h. Cell lysates were prepared as described in material and methods. Samples were run on denaturing gels. The membranes were then blocked with 5% non-fat milk in PBS for 1h, followed by incubation with primary rabbit polyclonal antibody against CERK (abcam, ab 155061) in 1: 500 dilution at 4°C overnight. The blots were then washed four times with TBS and incubated for 2h with HRP-conjugated secondary antibody (Promega, Madison, WI, USA). Immuno-reactive bands were developed using an Amersham ECL Plus Westren Blotting Detection System (GE Healthcare, Chicago, IL, USA) and visualized by Molecular Imager ChemiDoc MP Imaging Systems (Bio-Rad Laboratories, Hercules, CA, USA). CERK is shown in the upper panel with the lower panel representing house keeping gene Beta-actin.

C)

Supplementary Figure 3

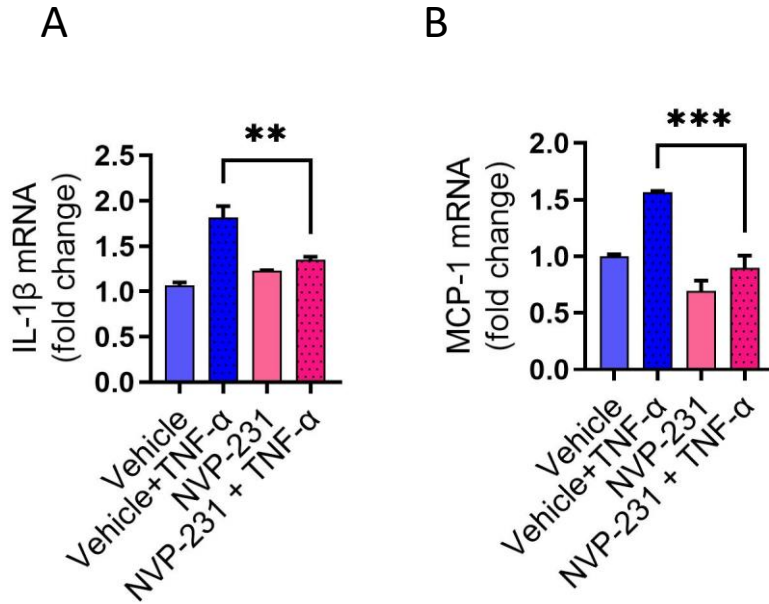


Figure S3. IL-1 β and MCP-1 gene expression by TNF- α activated monocytes are suppressed by CERK inhibition.

Monocytic cells were pretreated with CERK inhibitor (NVP-231: 12nM) or vehicle for 1h and then incubated with TNF- α for 4h. Cells were harvested for total RNA isolation. Gene expression of IL-1 β (**A**) and MCP-1 (**B**) was determined by real time RT-PCR. All data are expressed as mean \pm SEM ($n \geq 3$). ** $p \leq 0.01$, *** $p \leq 0.001$ versus vehicle + TNF- α .

Supplemental Figure 4

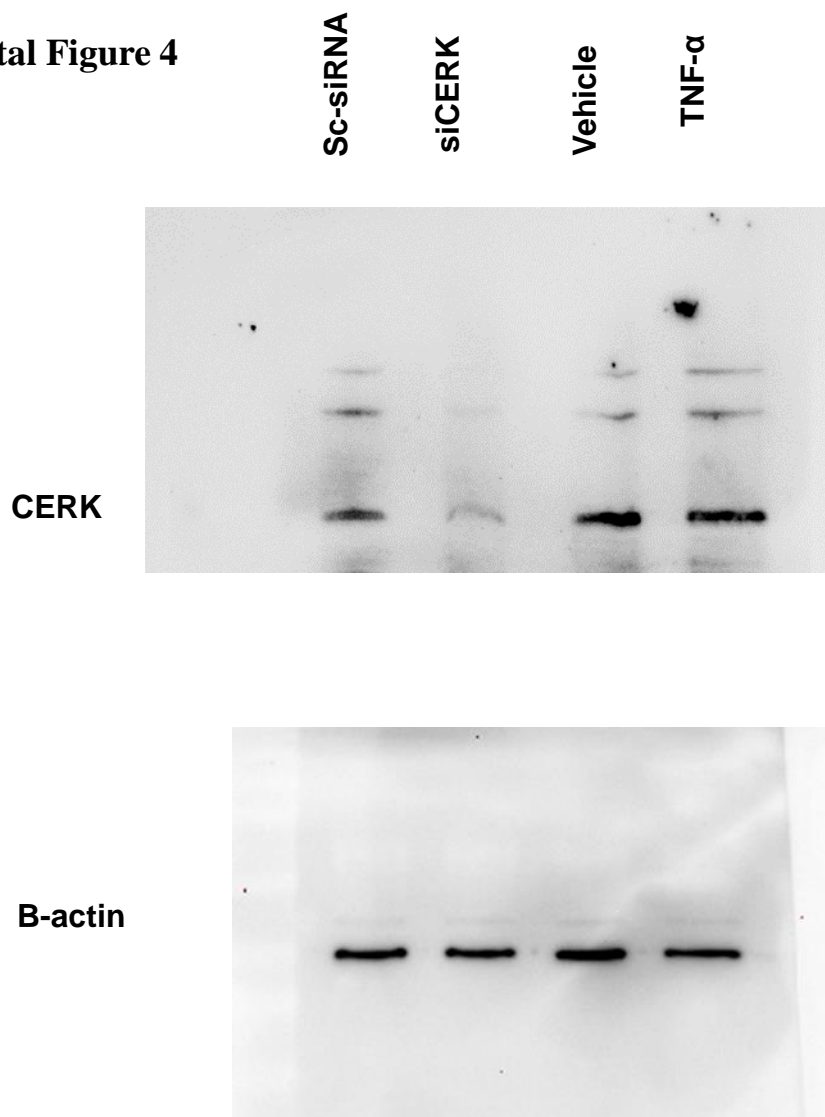
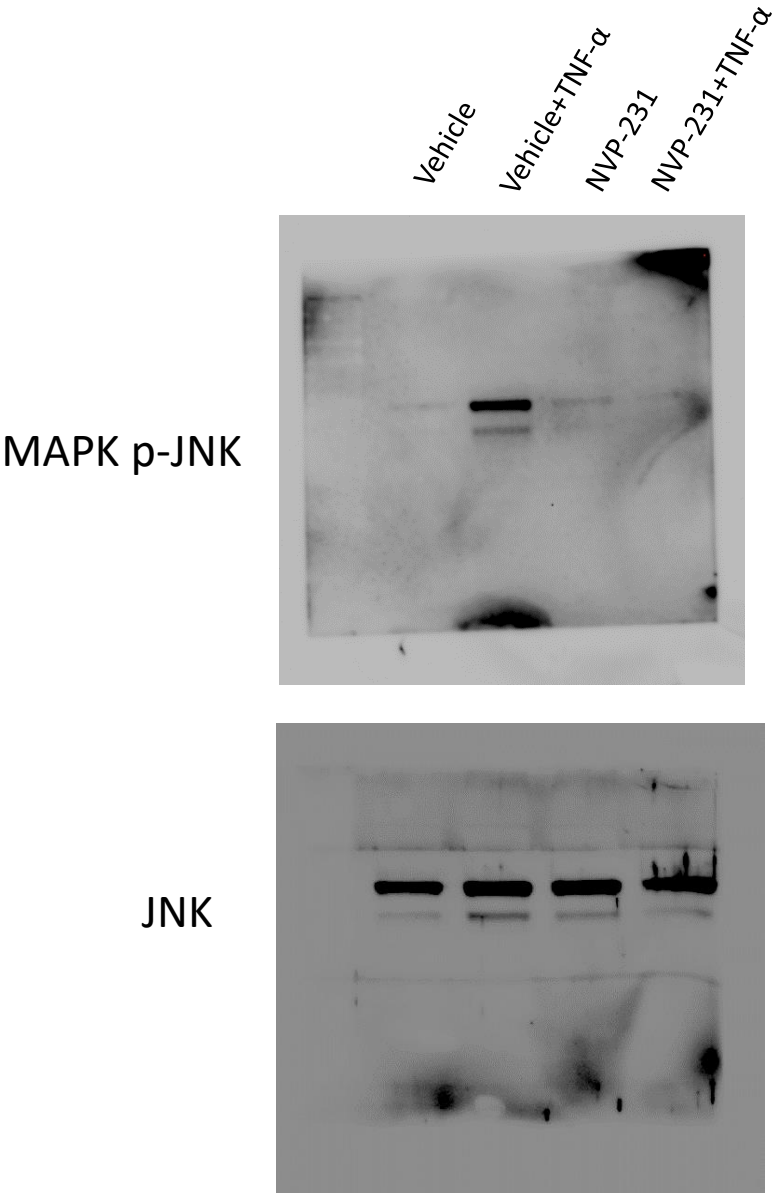


Figure S4. SiRNA transfection efficacy. THP-1 monocytic cells were transfected with scrambled-siRNA (negative control; NC) or CERK siRNA as described in material and methods. Cell lysates were prepared as described in material and methods. Samples were run on denaturing gels. The membranes were then blocked with 5% non-fat milk in PBS for 1h, followed by incubation with primary rabbit polyclonal antibody against CERK (abcam, ab 155061) at 1: 500 dilution or B-actin at 1: 1000 dilution (Cell Signaling Technology, Inc) at 4°C overnight. The blots were then washed four times with TBS and incubated for 2h with HRP-conjugated secondary antibody (Promega, Madison, WI, USA). Immuno-reactive bands were developed using an Amersham ECL Plus Westren Blotting Detection System (GE Healthcare, Chicago, IL, USA) and visualized by Molecular Imager ChemiDoc MP Imaging Systems (Bio-Rad Laboratories, Hercules, CA, USA). CERK is shown in the upper panel with the lower panel representing house keeping gene Beta-actin.

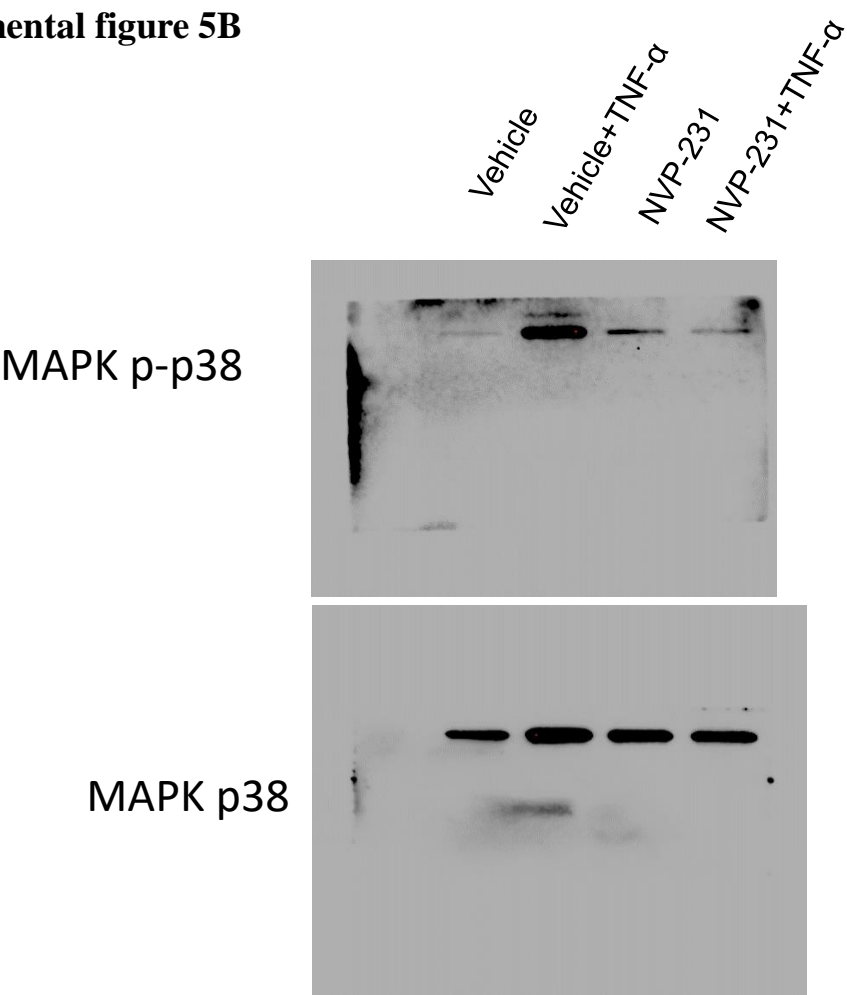
Supplemental Figure 5A-C (full western blot images for Figure 7):

THP1 monocytic cells were pretreated with CERK inhibitor (NVP-231: 12nM) and then incubated with TNF- α . Cell lysates were prepared as described in material and methods. Samples were run on denaturing gels. Immuno-reactive bands were developed using an Amersham ECL Plus Westren Blotting Detection System (GE Healthcare, Chicago, IL, USA) and visualized by Molecular Imager® ChemiDoc™ MP Imaging Systems (Bio-Rad Laboratories, Hercules, CA, USA). **(A)** Phosphorylated proteins of SPAK/JNK, **(B)** p38 and **(C)** NF-kB are shown in the upper panels with the lower panel representing respective total proteins.

Supplemental figure 5A



Supplemental figure 5B



Supplemental figure 5C

